Br J Vener Dis 1980;**56**:235-8.

Bacterial interference of Neisseria gonorrhoeae by α-haemolytic streptococci

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SUMMARY Fifty pharyngeal isolates of α-haemolytic streptococci were tested against 20 cervical isolates of *Neisseria gonorrhoeae* for bacterial interference in vitro using the lawn-spotting method. Forty-seven (94%) isolates of streptococci showed inhibitory activity toward *N gonorrhoeae,* although nine of these were inhibitory to only one isolate of *N gonorrhoeae.* Isolates of *N gonorrhoeae* varied widely in their sensitivity to streptococci; the most sensitive were inhibited by 40 isolates of streptococci and the least sensitive by only 14 isolates. Species of *Streptococcus* found to inhibit growth of *N gonorrhoeae* were *S mitis, S MG intermedius, S sanguis II, S mutans,* and *S morbillorum.*

Introduction

In a previous study of cervical flora in women with recent sexual contact with men infected with *Neisseria gonorrhoeae,*[1] one woman, who had a negative culture result for *N gonorrhoeae,* was found to have a high population of α-haemolytic streptococci in the cervix. This isolate of α-haemolytic streptococci inhibited *N gonorrhoeae* in vitro. Since α-haemolytic streptococci are known to inhibit a variety of micro-organisms,[2] including *Neisseria* species,[3] we decided to survey a larger number of strains of each of these organisms for evidence of bacterial interference and of their prevalence and to establish whether or not there were differences in susceptibility between various isolates of *N gonorrhoeae.*

Materials and methods

BACTERIAL STRAINS

Fifty isolates of α-haemolytic streptococci were obtained from pharyngeal swabs from healthy men and women between the ages of 20 and 50 by culture on Casman's sheep blood agar. Single-colony isolates were selected on the basis of their α-haemolytic activity; frequently more than one strain, as differentiated by colonial morphology, was isolated per person. Isolates were identified by the following tests using the scheme of Facklam[4]: optochin sensitivity and bile solubility, bile esculin hydrolysis, growth in 6·5% NaCl, growth in 5% sucrose agar and broth, hippurate hydrolysis, and use of mannitol, lactose, inulin, esculin, and raffinose. Cultures were frozen at −4°C in trypticase soy broth (Difco) containing 20% glycerol and also maintained on Casman's sheep blood agar (Baltimore Biological Laboratories, Maryland, USA) at 4°C.

Isolates of *N gonorrhoeae* were obtained from the Houston Public Health Laboratory from cervical cultures of patients reporting with suspected gonococcal infections. Cervical swabs were immediately plated on to Thayer-Martin medium and incubated in CO_2 for 18-24 hours. Oxidase-positive colonies of Gram-negative diplococci were presumptively identified as *N gonorrhoeae,* subcultured on to chocolate agar, and incubated at 37°C in CO_2 jars. After 18-24 hours' incubation, growth was used immediately for bacterial interference experiments (first subculture). This growth was also suspended in trypticase soy broth (BBL) containing 20% glycerol and stored at −70°C. Chocolate agar was prepared by adding 5% sheep blood to BBL blood agar base and heating in a boiling waterbath for 10 minutes.

BACTERIAL INTERFERENCE

Uniform suspensions were made from the first subculture of *N gonorrhoeae* in phosphate-buffered saline, pH 7·2, measuring turbidity visually against McFarlane tube 2. Lawns were prepared by swabbing the suspension on to the surface of chocolate agar

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Received for publication 4 June 1979

plates. Isolates of α-haemolytic streptococci were incubated overnight on Casman's blood agar and spotted directly on to the surface of the lawns; the plates were then incubated in CO_2 jars at 37°C for 18-24 hours. Zones of inhibition of growth of *N gonorrhoeae* were measured from the edge of the streptococcal growth for the purpose of checking the consistency of the observations in repeated trials.

Results

Fig 1 shows zones of inhibition of growth of *N gonorrhoeae* around inoculation of α-haemolytic streptococci, demonstrating varying degrees of inhibition; zones varied between 1 and 5 mm. The inoculum was not quantitated, but certain isolates of α-haemolytic streptococci were found repeatedly to produce larger zones of inhibition than others.

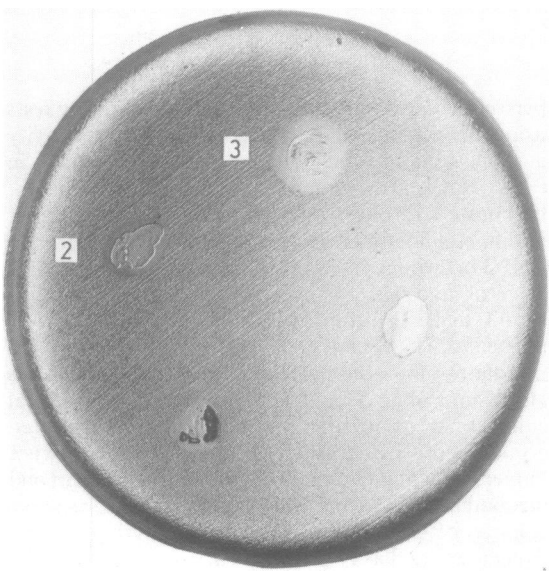

FIG 1 *Four different isolates of α-haemolytic streptococci spotted on lawns of N gonorrhoeae showing varying degrees of inhibition of growth.*

Not all isolates of α-haemolytic streptococci inhibited *N gonorrhoeae;* similarly, different isolates of *N gonorrhoeae* varied in their sensitivity to α-haemolytic streptococci. This is shown in fig 2, in which 50 isolates of streptococci have been arranged along the ordinate in order of their decreasing inhibitory activity, measured by the number of strains of *N gonorrhoeae* they inhibit. Isolates of *N gonorrhoeae* are arranged along the abscissa in order of their decreasing sensitivity to α-haemolytic

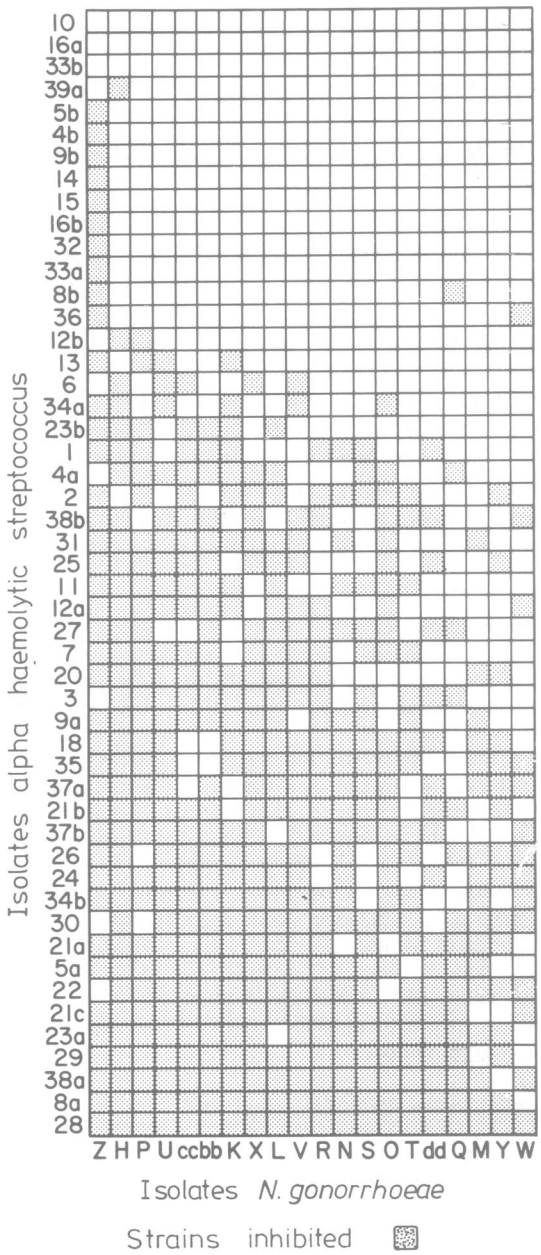

FIG 2 *Inhibition pattern of 20 isolates of N gonorrhoeae by 50 isolates of α-haemolytic streptococci.*

streptococci. Only three of the 50 isolates of α-haemolytic streptococci were without inhibitory activity to any of the 20 isolates of *N gonorrhoeae* tested; a further nine inhibited only one isolate of *N gonorrhoeae* (fig 2). On the other hand, 18 (36%)

isolates of α-haemolytic streptococci inhibited at least 16 (80%) isolates of *N gonorrhoeae,* even though only one isolate of streptococci inhibited all 20 isolates of *N gonorrhoeae.*

Isolates of *N gonorrhoeae* varied greatly in their sensitivity to the inhibitory activity of α-haemolytic streptococci; the strain designated Z was inhibited by 40 of the isolates of streptococci whereas strain W was inhibited by only 14 (fig 2). It was interesting that some of the isolates of streptococci which inhibited the largest number of isolates of *N gonorrhoeae* (that is, 22) did not inhibit the growth of the most sensitive strain (Z).

The distribution of species of the pharyngeal isolates of α-haemolytic streptococci are shown in table I. *S mitis* was the predominant species, followed by *S sanguis* II and *S MG intermedius.* Inhibitory activity of streptococcal isolates toward *N gonorrhoeae* was not associated with any single species; both active and inactive strains of all species listed in table I were found. Table II shows the frequency of isolation of different strains within species of streptococci showing inhibition toward *N gonorrhoeae.* Of *S mitis* strains isolated, 63·6% inhibited more than 50% of strains of *N gonorrhoeae* tested; 50% of *S MG intermedius* strains had similar activity whereas only 22·2% of *S sanguis* II were inhibitory.

TABLE I *Distribution of species of 50 pharyngeal isolates of α-haemolytic streptococcus*

Species	*% Incidence*
Streptococcus mitis	59·10
Streptococcus sanguis II	20·50
Streptococcus MG intermedius	11·40
Streptococcus morbillorum	4·50
Streptococcus mutans	2·00
Streptococcus sanguis I	2·00

TABLE II *Inhibitory activity of different species of streptococci toward N gonorrhoeae*

	% of strains isolated showing	
Species	*Low activity**	*High activity†*
S mitis	36·4	63·6
S MG intermedius	50·0	50·0
S sanguis II	77·8	22·2

*Inhibitory to <50% of strains of *N gonorrhoeae* tested
†Inhibitory to >50% of strains of *N gonorrhoeae* tested

Discussion

Although the α-haemolytic streptococci used in these experiments were a heterogeneous group, it appears that a high percentage of pharyngeal streptococci are capable of inhibiting *N gonorrhoeae.* It is interesting to speculate whether or not this antagonism occurring in vitro could provide protection against infection in vivo. Although inhibition of *N gonorrhoeae* has been demonstrated in vitro by a number of organisms—*Candida albicans,*[5] *Staphylococcus epidermidis,*[6] non-haemolytic streptococcus,[7] and various enteric Gram-negative bacteria[8]—attempts to show that interference provides protection are few. To have a protective effect, α-haemolytic streptococci would need to occur frequently in the normal flora and in large numbers. In a review of seven studies on the normal flora of the vagina and cervix,[9] the isolation rate for *Streptococcus viridans* or α-haemolytic streptococci varied from 0 to 53%. In more recent quantitative studies, α-haemolytic streptococci have still occurred in varying frequencies but in large numbers.[10-12] The true incidence of α-haemolytic streptococci may not yet be accurately recorded, since the use of selective media for vaginal cultures would inhibit its growth; furthermore, an overgrowth of fast-growing bacteria, such as *Proteus* spp or *Escherichia coli,* would mask its presence.

In our previous study,[1] a protective effect by α-haemolytic streptococci against gonococcal colonisation was apparent in only one patient. Recent attempts to relate the presence of certain groups of organisms in the normal cervical flora to protection against *N gonorrhoeae* have shown that *Staph epidermidis* isolates were most active against *N gonorrhoeae* in vitro,[13] although no correlation was looked for between the presence of *Staph epidermidis* and infection with *N gonorrhoeae.* Saigh *et al*[14] attempted to relate the presence of antagonistic organisms, which were mostly non-haemolytic streptococcal species, staphylococcal species, and *Lactobacillus* spp, to infection with *N gonorrhoeae.* The presence of *Lactobacillus* spp was found to correlate with lack of acquisition of *N gonorrhoeae,* which was interpreted as a cause-and-effect relationship. The differences in the isolation rate of *Lactobacillus* spp were also noted in our study of normal cervical flora in relation to gonorrhoea,[1] but this difference was most obvious between the two population groups, public clinic and private patients. Our interpretation was not necessarily one of cause and effect; however, we believed that the lack of *Lactobacillus* spp in the cervical flora of clinic patients infected with *N gonorrhoeae* was either a result of other factors, such as recurrent episodes of gonorrhoea and treatment with antibiotics eliminating *Lactobacillus* spp, or due to the fact that *N gonorrhoeae* itself might have an inhibitory effect on *Lactobacillus* spp—an effect that we were able to demonstrate in one instance.

While α-haemolytic streptococci may not occur in cervical flora sufficiently often or in great enough numbers to provide protection from gonorrhoea, it may play a role in the prevention of gonococcal pharyngitis. Pharyngeal colonisation by *N gonorrhoeae* usually affects less than 10% of populations attending venereal disease clinics.[15] When certain specialised groups are selected, such as homosexual men who claim to have had oral contact with partners infected with *N gonorrhoeae,* the incidence is higher.[16 17]

The authors wish to thank Mrs Ann Gould for the clinical isolates of *N gonorrhoeae* used in this study and Miss Judith Singer for technical assistance.

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